

<https://helda.helsinki.fi>

The molecular genetics and neurobiology of developmental dyslexia as model of a complex phenotype

Kere, Juha

2014-09-19

Kere , J 2014 , ' The molecular genetics and neurobiology of developmental dyslexia as model of a complex phenotype ' , Biochemical and Biophysical Research Communications , vol. 452 , no. 2 , pp. 236-243 . <https://doi.org/10.1016/j.bbrc.2014.07.102>

<http://hdl.handle.net/10138/217896>

<https://doi.org/10.1016/j.bbrc.2014.07.102>

cc_by_nc_nd

publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.



Review

The molecular genetics and neurobiology of developmental dyslexia as model of a complex phenotype



Juha Kere*

Department of Biosciences and Nutrition, Centre for Innovative Medicine, Karolinska Institutet, Stockholm, Sweden
Molecular Neurology Research Program, University of Helsinki, Folkhälsan Institute of Genetics, Helsinki, Finland

ARTICLE INFO

Article history:

Received 27 June 2014

Available online 28 July 2014

Keywords:

Reading disability
Neuronal migration
Cilia function
White matter
Cognitive function

ABSTRACT

Among complex disorders, those concerning neuropsychiatric phenotypes involve particular challenges compared to disorders with more easily distinguished clinical signs and measures. One such common and unusually challenging phenotype to disentangle genetically is developmental dyslexia (DD), or reading disability, defined as the inability to learn to read and write for an otherwise normally intelligent child with normal senses and educational opportunity. There is presently ample evidence for the strongly biological etiology for DD, and a dozen susceptibility genes have been suggested. Many of these genes point to common but previously unsuspected biological mechanisms, such as neuronal migration and cilia functions. I discuss here the state-of-the-art in genomic and neurobiological aspects of DD research, starting with short general background to its history.

© 2014 The Author. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

Contents

1. Introduction	236
2. Evidence for biological background of DD	237
3. Genetic linkage studies identified loci for dyslexia	237
4. First candidate susceptibility genes for DD	238
5. DYX1C1 and CYP19A1	238
6. DCDC2 and KIAA0319	239
7. ROBO1	239
8. C2Orf3 and MRPL19	239
9. Neuronal migration defects in rat models caused by silencing of three DD genes	239
10. Human brain imaging revealed effects on white matter and axonal connections	239
11. Biochemical and cell biological functions of DD genes	240
12. Conclusion	241
Acknowledgments	241
References	241

1. Introduction

Developmental dyslexia (DD) is one of many often co-occurring learning disabilities, but typical of it is the stark contrast between a child's overall performance and the distinct problems in learning to

read and write. An early description of DD by Bastian [1] has documented that nicely, but even though these authors made a distinction between developmental and acquired (e.g., following brain trauma), the often familial clustering waited for later documentation. Besides occasional notes in the early 1900's, first Norrie in 1939 (cited in [2]) reported familial clustering in nearly all cases, and Hallgren's study in 1950 [3] of 116 index individuals and 160 affected family members made a compelling case. Hallgren (1950) also suggested dominant inheritance as the most plausible mode of inheritance.

* Address: Department of Biosciences and Nutrition, Centre for Innovative Medicine, Karolinska Institutet, Stockholm, Sweden.

E-mail address: juha.kere@ki.se

Importantly, DD can occur in children with perfectly normal overall intellect, but in the past they were often unfortunately labeled as “backward” or “stupid” [4]. There is still room today for improvement for how schools and parents can recognize and diagnose DD early enough, get appropriate help and training for a child, and prevent the untoward feeling of being different and becoming socially handicapped. The goal should be to allow every child to reach his or her full individual intellectual potential.

Indeed, it is the specificity of the defect in learning that makes DD an unusually interesting phenotype to study and understand. But not only is DD interesting from the neuropsychological point of view; DD involves one of the very specific skills that distinguishes us humans from the other primates that cannot learn character-based coding and decoding. Unsurprisingly, there are likely common threads between language development and reading and writing, as many dyslexic children have a history of delayed language development as well. Looking beyond our species, the developmental mechanisms that have allowed language, reading and writing to evolve in humans are unlikely to be fundamentally different from mechanisms that may have been adapted to other tasks in other organisms. Thus, an understanding of the molecular and neurobiological mechanisms of DD might more generally also teach us something about cognition, the developmental processes of the brain, and the specific evolution of the human brain.

2. Evidence for biological background of DD

Even before the advent of genomic studies, multiple converging lines of evidence have suggested that DD has an early developmental and biological etiology. The familial occurrence with even apparent dominant patterns of inheritance suggested genetic background early on [2,3]. These studies have been expanded to observations on twins that have supported multifactorial genetic etiology rather than simple dominant inheritance in most cases [5]. Importantly, these studies have supported a strong genetic effect, reaching 70–80% for different reading and related measures, in contrast to modest classroom or other environmental effects. Specific loci have been mapped by genetic linkage methods in exceptionally large pedigrees, providing strong evidence of dominant gene effects in some families [6,7].

Other lines of evidence have studied brain event-related potentials in children of dyslexic parents. The results have indicated early biological effects already in newborn babies, long before reading and writing skills can develop [8] and further extended into associations to poor verbal memory skills at age 5 before the development of reading skills [9]. Again, the early onset of related problems lend support for the notion of biological rather than environmental influence at the bottom of DD.

Brain imaging approaches employing magnetic resonance imaging (MRI) found differences in white matter microstructure bilaterally in temporo-parietal regions between DD and normal readers [10]. Independent studies using positron emission tomography (PET) to measure brain activation patterns in DD and normal readers speaking different languages found common correlates in all [11]. More specifically, there were common brain areas activated in all individuals and particular areas in the left temporal and occipital gyri that were significantly less activated in DD than in normal readers. Interestingly, later PET studies involving Chinese participants using a logographic writing system found also brain areas with reduced activation in DD, but the areas were different from those using alphabetic writing [12].

Even though the biological correlates of brain structure and activation appear in the same anatomical regions irrespective of language, their differences necessitate emphasis on different aspects and measures for DD when testing children and

establishing diagnostic criteria. The learning profiles for spelling and writing may be very different in highly orthographic languages (such as Finnish) in comparison to languages with irregular spelling (such as English or French). The variation in testing and diagnostic criteria obviously makes it more difficult to combine subjects from different countries, and may increase heterogeneity between study participants. Combined with genetic differences between populations, the cumbersome diagnostics, and heterogeneity of criteria may explain at least partially the lack of successful large-scale genetic association studies as of yet. Typically, such studies require beyond ten thousand participants to yield strong association results for genetic loci with modest risk effects.

Thus it may not be surprising that our knowledge of specific susceptibility genes in DD is still limited to such loci that have been implicated by single-gene strategies, such as genetic linkage studies in unusual large dominant families and subsequent targeted association studies as well as chromosome translocations or chromosomal deletions associated with individuals with DD. I will in the next paragraphs present the first susceptibility genes implicated in DD and follow them up with neurobiological, cell biological and neuroimaging data that have illuminated the possible biological mechanisms of DD.

The literature on the molecular genetics and neurobiology of DD is already so extensive that this review cannot cite all the relevant studies. The focus is kept on the identification and first implications of the first DD susceptibility genes. For complementary information on DD, the reader may look for other recent reviews [13,14].

3. Genetic linkage studies identified loci for dyslexia

The diagnosis of DD is not based on a simple laboratory test, but depends on the combination of personal history, assessment of cognitive skills, and sophisticated neuropsychological testing [15,16]. There is unquestionable variation in the degree of DD and also distinct phenotypic heterogeneity, both of which contribute to difficulties in designing and performing genetic studies.

As in many complex disorders, the first attempts to identify genetic loci influencing susceptibility were based on genetic linkage mapping in unusually large families with dominant inheritance patterns or multiple small families (introducing the risk of genetic heterogeneity). Table 1 lists those loci that have been recognized as replicated by the Human Gene Nomenclature Committee that has also named them as DYX1 through DYX9. It is worth noting that even though the genetic linkage studies have been based on families collected from different countries (and thus speaking different languages), the results of genetic mapping have been largely consistent.

In the early 2000's, these loci became also the targets of positional cloning studies with various strategies. The first candidate susceptibility genes for DD were identified based on studies of rare chromosomal translocations localizing within the implicated genetic loci on chromosome 15 (DYX1, gene DYX1C1) [17] and chromosome 3 (DYX5, gene ROBO1) [18]. Parallel efforts employed genetic fine-mapping based on assessing associations at increasing resolution, and yielded two candidate DD genes on chromosome 6 (DYX2, genes DCDC2 and KIAA0319) [19–22], chromosome 2 (DYX3, genes C2Orf3 and MRPL19) [23] and somewhat later on chromosome 18 (DYX6, genes MC5R, DYM and NEDD4L) [24,25]. A cluster of additional four genes was suggested on the basis of a submicroscopic deletion of chromosome 21 (genes PCNT2, DIP2A, S100B, and PRMT2) [26], even though this locus had not been previously recognized by genetic linkage studies. For many of the genetically linked loci, there is still no further evidence of specific genes, which may be explained either as the absence of fortuitous

Table 1
Genetic loci for DD that have been recognized as replicated by the Human Gene Nomenclature Committee (<http://www.genenames.org/>). The loci are named in the order of discovery as DYX1 through DYX9.

Gene	Chromosome, locus	Degree of reliability	Proposed mechanism(s)
DYX1C1	15q, DYX1	Replicated in many studies	Neuronal migration in embryonal period, regulation of estrogen signaling, ciliary function
ROBO1	3p, DYX5	Replicated	Regulation of axonal and dendritic growth
DCDC2	6p, DYX2	Replicated in many studies	Neuronal migration in embryonal period, ciliary function
KIAA0319	6p, DYX2	Replicated in many studies	Neuronal migration in embryonal period
C2Orf3, MRPL19	2p, DYX3	No replications yet	No known mechanism
PCNT, DIP2A, S100B, PRMT2	21q, no locus named	No replications yet	No known mechanism
MC5R, DYM, NEDD4L	18p, DYX6	No replications yet	No known mechanism
DGK1	7q, no locus named	No replications yet	No known mechanism
CYP19A1	15q, DYX1	No replications yet	Regulation of estrogen signaling
No gene implicated yet	6q, DYX4	Genetic linkage only, no gene	No known mechanism
No gene implicated yet	11p, DYX7	Genetic linkage only, no gene	No known mechanism
No gene implicated yet	1p, DYX8	Genetic linkage only, no gene	No known mechanism
No gene implicated yet	Xq, DYX9	Genetic linkage only, no gene	No known mechanism

chromosomal abnormalities facilitating gene identification or possibly as false mapping signals.

4. First candidate susceptibility genes for DD

The first susceptibility genes for DD possess different structural properties and belong to different gene families. Therefore, it has been surprising to observe that the mechanisms to which they seem to connect functionally have merged and suggested a small number of specific pathways of relevance in the development of the brain. In the next section I will discuss briefly the genes and the rather sparse data that their structures provided. It was first the functional studies that provided merging results discussed in later paragraphs.

5. DYX1C1 and CYP19A1

The first DD susceptibility gene was identified by study of a family with dyslexic father and three out of four children, all carrying also a balanced chromosome translocation t(2;15)(q11;q21). The DYX1 locus had previously been mapped to chromosome 15q21, and thus we hypothesized that the chromosome translocation breakpoint was in the immediate vicinity of a DD susceptibility gene. We then cloned a new gene, first called EKN1 and later renamed DYX1C1, disrupted by the translocation breakpoint. DYX1C1 was found expressed in neurons in brain samples, but its structure did not provide suggestions toward its specific biochemical role beyond the cognate tetratricopeptide repeat and heat-shock protein domains [17].

To verify the role of DYX1C1 in DD, several replication association studies have been undertaken by investigators around the world. Because the diagnosis of DD requires thorough assessment, many studies are based on rather small sets of participants, resulting in compromised power to detect genetic associations. Understandably, then, the replication analysis results are highly variable, with several studies reporting confirmation of genetic association, but also many studies not detecting it (importantly, such studies have no power to reject the possibility of genetic association either; rejection of the hypothesis of association would require even larger data sets). A compilation of these studies and their most significant association results are summarized in Fig. 1. There is a tendency of positive replications more often in larger data sets; however, the associations reported may be to different markers and sometimes in opposite directions in different studies. Remarkably, positive associations have been reported not only for users of the alphabetic writing system, but also for Chinese. One can conclude that it is likely that DYX1C1 plays a general

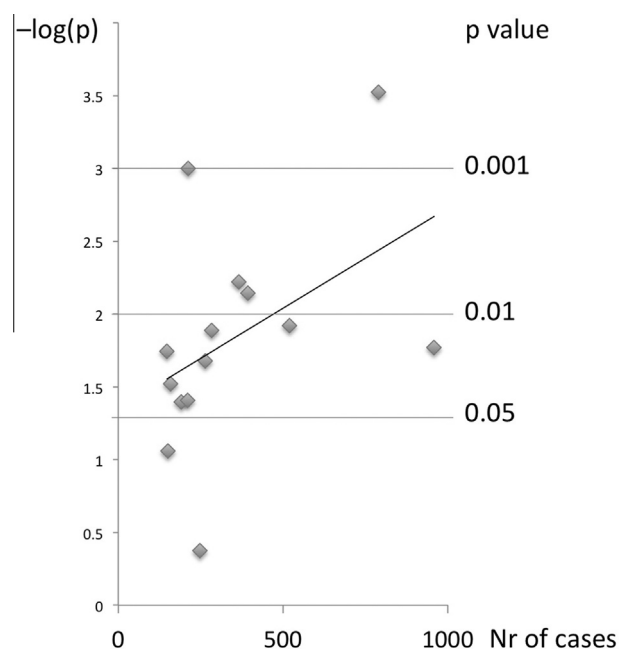


Fig. 1. A compilation of DYX1C1 replication studies and their most significant association results. The studies are plotted according to the number of DD cases included in each study and the most significant association *p* value reported, irrespective of which marker yielded the result. Most studies would appear underpowered by today's standards, but there is a tendency to obtain significant association results in larger studies. A complete list of references to the studies is available from the author upon request.

role in DD susceptibility, but the effect is weak and there may be population heterogeneity.

A second, different translocation patient with DD and a breakpoint in chromosome 15q, in the broad DYX1 locus, had been also identified and became later the topic of detailed study [27,28]. In this patient, the translocation breakpoint was clearly distinct from the DYX1C1 translocation, mapping 6–8 Mb more proximally. The breakpoint was found to localize near the brain promoter of CYP19A1 encoding the aromatase enzyme. Aromatase catalyzes the conversion of testosterone to estrogen in many tissues, notably the reproductive organs, but also in the brain. Excitement for aromatase was increased because it had been widely studied as a gene that regulates songbird singing behavior, another form of vocal signaling besides language. The brain promoter of CYP19A1 turned out to be even more highly conserved across species than its exons,

suggesting a highly constrained role in evolution. Moreover, a comparison of primate sequences identified a single base different between human and other primates, and this variation was shown to cause a gain of transcription factor binding to the human sequence. Genetic association studies were then performed on several cohorts recruited to study DD and language, and highly significant associations were detected to language and reading quantitative traits [28]. A study of the direct effects of aromatase function on neurite outgrowth in undifferentiated neurons using rat embryonal day 17 hippocampal neurons as a model indicated that aromatase-dependent conversion of testosterone to estradiol enhanced neurite outgrowth. Finally, aromatase knock-out (ArKO) mice showed signs of cortical disorganization. The neuronal density in cortical areas was significantly increased at embryonic day 17.5, and even in mature mice, the cortical layers II/III had an increased neuronal density in ArKO mice. Taken together, these results suggested a distinct role for CYP19A1 and aromatase in the development of the brain areas relevant to the ability to learn written and spoken language [28].

6. DCDC2 and KIAA0319

The second locus for DD to be mapped (DYX2) was identified on chromosome 6p21–p22. Several research groups then embarked on a race to find the corresponding gene. DYX2 was first refined to include five genes within less than 600 kb [29]. Four research groups working independently implicated surprisingly two different genes, DCDC2 and KIAA0319, within this region. Each gene was supported by two research groups and rejected by the other two. DCDC2 was identified independently in parallel by a U.S. group and a German-Scandinavian team [19,20], and the studies failed to detect any support for the other gene. In contrast, KIAA0319 instead of DCDC2 (with specific negative findings) was supported by two teams from the U.K. [21,22].

Again, the initial identification of two genes with strong supporting and rejecting evidence spurred several replication attempts, with both positive and negative results similar to DYX1C1. One may conclude from the bulk of these studies that most likely both genes play a role in DD susceptibility, but the genetic effect may not be any stronger than that of DYX1C1.

7. ROBO1

The ascertainment of a large three-generation family that included 21 individuals with DD apparently segregating in the autosomal dominant mode provided an excellent starting point for a genetic study. Genetic linkage analysis identified a dominant locus on chromosome 3 (named subsequently DYX5) and confirmed that in nearly all individuals with DD the phenotype was likely to have the same, common origin [7,30].

Later studies confirmed linkage to this locus in families from the U.S. and U.K. [24], and curiously, perhaps reflecting similar mechanisms, the same locus was detected in a study of speech-sound disorder in U.S. families [31]. The linkage signal implicated, however, a rather broad genomic region, especially because the locus was located near the centromere (where recombinations are rare and thus one cM may correspond to several Mb of DNA), making it difficult to implicate a single gene. We identified an individual with DD and a chromosome translocation involving the DYX5 locus, and worked out the translocation breakpoint by using genomic clones in fluorescence in situ hybridization experiments [18]. The translocation breakpoint turned out to disrupt the ROBO1 gene between its two known promoters, thus likely hampering its normal regulation. ROBO1 is one of four human orthologs for the *Drosophila* roundabout, or *robo* gene that was found critical for normal axon crossing between the brain halves (hence the name;

axons turned back and forth in the midline of the developing mutant fruit flies). Later studies had also shown that *robo* participates in dendrite guidance [32]. *Robo1* knockout mice die at birth, have small or absent corpus callosum and hippocampal commissure, and have also neuronal migration defects in the forebrain [33]. Such functions of the gene were immediately interesting considering the pathogenesis of DD.

But was there any connection to ROBO1 in the large family that first implicated linkage to DYX5? We could show that the expression of the ROBO1 allele that segregated with DD was attenuated compared to the other allele in several studied individuals (however, the assay could only be done using blood cells) [18].

8. C2Orf3 and MRPL19

A large Norwegian family with dominant pattern of inheritance for DD had been used to map the DYX3 locus on chromosome 2p11–p15 [6]. We confirmed genetic linkage to the same locus in some Finnish families, and performed then fine-mapping studies in Finnish and German families [23]. Genetic associations narrowed down the locus to markers between two genes, C2Orf3 and MRPL19, that turned out to be coregulated. The genetic association remained unconfirmed until recently, when several large cohorts showed significant associations of this locus to general cognitive ability [34].

9. Neuronal migration defects in rat models caused by silencing of three DD genes

In parallel with the reporting of ROBO1 as a DD susceptibility gene, implicating axonal or dendrite guidance as possible pathogenic mechanisms, functional study of the DCDC2 gene suggested that neuronal migration may play a role [19]. Downregulation of rat *Dcdc2* during embryonal period using RNA interference hampered normal neuronal migration from the ventricular zone to cortex, leaving the cells to subcortical localizations. Surprisingly, then, similar results were quickly reported for both *Dyx1c1* and *Kiaa0319* in rats around gestational day 14 [35,36]. Dendritic growth and differentiation defects were also observed with silencing of *Kiaa0319* [36]. Correction of the silencing by transient overexpression restored normal neuronal migration in control experiments [19,35,36].

An enigmatic study by Albert Galaburda had reported microscopic cortical structure defects, cortical ectopias, in the brains of four deceased individuals with DD [37]. When two groups independently studied the fine-structure of adult rat brains with in utero silenced *Dyx1c1*, they reported similar cortical ectopias as those observed by Galaburda in DD [38,39]. Interestingly, thorough neurological characterization of rats with in utero silenced *Dyx1c1* revealed that they have as adults abnormal processing of auditory stimuli, insensitivity to auditory cues and also a spatial learning disability in water maze experiments [39].

10. Human brain imaging revealed effects on white matter and axonal connections

Early studies of DD using brain imaging techniques found microstructural alterations in white matter in temporo-parietal regions of the brain [10] and functional differences in brain regions activated during reading tasks, implicating a region that was less activated in DD [11]. Combining genotyping and structural brain imaging in healthy school-age children, Darki et al. [40] studied the possible effects of genetic variants in three genes, *DYX1C1*, *DCDC2* and *KIAA0319* in normal brains using magnetic resonance imaging (MRI). All three genes were found to have alleles that associated with white matter volume in temporo-parietal regions.

Furthermore, the regions affected by each of the three genes were partially overlapping, and tractography revealed that white matter tracts passing through the identified region connected the middle temporal gyrus with the inferior parietal lobe. The cortical projection of that position coincided with the region implicated as less activated in DD by the earlier study of Paulesu et al. [11] and might reflect the suggested poor connectivity [41]. Another study had detected that genetic variants within DCDC2 associated with differences in cortical morphology of healthy individuals, in particular in the gray matter [42]. Thus, the results interestingly merged imaging results from the pre-genetic era with studies of DD susceptibility gene effects. The finding suggested also that the same mechanisms may underlie the variability in reading skills in both normal readers and DD. Such conclusions had also emerged from genetic studies looking at DYX1C1 effects on quantitative reading measures in normal readers [43].

The complexity of possible mechanisms and pathways was then suggested by the imaging results on the C2Orf3/MRPL19 genes associated with DD and general cognitive ability [34]. MRI study based on the same participants as that looking for effects of DYX1C1, DCDC2 and KIAA0319 revealed that white matter volume varied depending on one variant in the C2Orf3/MRPL19 region bilaterally in the posterior part of the corpus callosum and the cingulum. Axonal projections connected analogous areas of the left and right hemispheres for postcentral gyrus, superior parietal lobule, precuneus, lateral occipital cortex and fusiform gyrus [34].

The functional effects of the suggested ROBO1 gene attenuation in the large DYX5-linked family from Finland were studied directly in family members using magnetoencephalography (MEG) to trace auditory responses to signals that could be tracked back to the receiving ear by frequency modulation [44]. The results suggested that ROBO1 regulates a phenomenon called interaural interaction for auditory signals. Most axons transferring auditory signals from each ear cross the midline at several levels to reach the contralateral auditory cortex, but a minority of axons project to the same, ipsilateral side with the receptive ear. Auditory cortices thus receive signals from both ears, and normally signals received at both ears suppress each other so that the cortical responses transmitted from the same side axons are attenuated by signals from the other side. Compared to controls, the attenuation by contralateral signals was absent or weak in those members of the DYX5-linked family who carried the DD allele. This phenomenon that depends on the crossing of auditory axons supported the role of ROBO1 as the DD gene. Furthermore, the expression levels of ROBO1 in blood samples, serving as a proxy for gene function, correlated significantly with the level of ipsilateral auditory suppression [44].

11. Biochemical and cell biological functions of DD genes

The identification of specific genes did not first prove very helpful for understanding their functions, with the exception of ROBO1 and DCDC2. ROBO1 was known as an axon guidance receptor regulating the connections between brain hemispheres, and DCDC2 was homologous to the DCX gene that had been implicated in causing lissencephaly, a severe brain malformation with the complete absence of gyri due to a severe neuronal migration defect. Importantly, mutations in several genes (DCX, LIS1, FLNA) can cause lissencephaly, but also a much milder neuronal migration defect presenting with epilepsy and other signs, periventricular heterotopia. DD might then represent the milder end with normal intelligence among a spectrum of disorders that result from defects in neuronal positioning or connectivity.

For DYX1C1 and KIAA0319, no such structural or functional clues were available, and thus any biological characterization

had to start hypothesis-free. For biochemical and genomic characterization, one can start by looking for “functional neighbours”, that is, genes and proteins that either regulate or are regulated by the gene in question, or for proteins that directly bind to the target protein. Obviously, gene and protein expression patterns can offer indirect clues, both at the level of organism and also at subcellular localization level. All these approaches have been used to elucidate the functions of DD susceptibility genes.

In an early study, Tapia-Páez et al. [45] identified transcription factors that would regulate DYX1C1 expression and more specifically, in an allele-dependent manner, considering that some of the first SNPs found associated localized to the 5' or promoter part of DYX1C1. Protein capture experiments using genomic probes corresponding to the 5' parts of DYX1C1 implicated three transcription factors, TFII-I, PARP1 and SFPQ, that can form a complex regulating DYX1C1 expression. Interestingly, even though these factors possess many regulatory functions in cells, animal models had suggested that some of these factors have functions in memory and brain development.

Altogether two leads suggest that estrogen signaling may play a role in dyslexia: the genetic implication of CYP19A1 in language development [28] and the functional implication of DYX1C1 protein in interactions with estrogen receptors α and β (encoded by the genes ESR1 and ESR2, respectively). Massinen et al. [46] showed that the DYX1C1 protein can regulate the estrogen receptors that are important also for brain development. ESR2^{-/-} mice display abnormal neuronal migration and increased apoptotic neuronal death [47,48]. ESR1 and ESR2 are both needed for cognitive functions and spatial learning in mice [49], and hippocampal synaptic plasticity and hippocampus dependent memory functions may depend on ESR2 mediated estrogen effects [50]. DYX1C1 protein complexes with ESR1 or ESR2 were detected in neurites of primary rat hippocampal neurons, compatible with a role in rapid estrogen signaling [46].

Recent studies have brought especially cilia and their functions at the forefront of understanding mechanisms of DD. First, DCDC2 was shown to have an effect on the length and signaling of primary cilia in neurons [51]. Overexpression of DCDC2 in rat hippocampal neurons increased ciliary length and activated Shh signaling, whereas downregulation of Dcdc2 expression induced Wnt signaling. The functional effect of DCDC2 was well conserved over a broad range of species, as overexpression of human DCDC2 (or its closest orthologue) in *C. elegans* caused an abnormal neuronal phenotype that was only observed in ciliated neurons [51]. Further evidence for the involvement of not only DCDC2 in ciliary functions but two other DD susceptibility genes, DYX1C1 and KIAA0319 as well came from bioinformatics analysis [52]. Finally, recent direct evidence by us [53] and others [54] revealed that the zebrafish ortholog *dyx1c1* is essential for normal cilia development in many ciliated organs, and cilia in morpholino downregulated fish showed missing dynein arms as well as situs inversus, kidney cysts, hydrocephalus and other malformations. Rare patients with compound heterozygous mutations of DYX1C1 were reported with primary ciliary dyskinesia and a functional ciliary phenotype was found in *Dyx1c1* knockout mice [54].

These results connect then well to findings that primary cilia are crucial for cortical morphogenesis as revealed by mouse *cobblestone* mutants that have subpial heterotopias in the forebrain, defects in the formation of the choroid plexus, cortical hem and hippocampus, and other anomalies [55]. Other studies point to role of cilia in coordinated migration and placement of interneurons and projection neurons. Live imaging of interneuronal cilia revealed that migrating cells have highly dynamic primary cilia, and the guidance cue receptors localize to interneuronal primary cilia. Expression of *Arl13b* variants known to cause Joubert syndrome, a clinically and genetically heterogeneous group of disorders

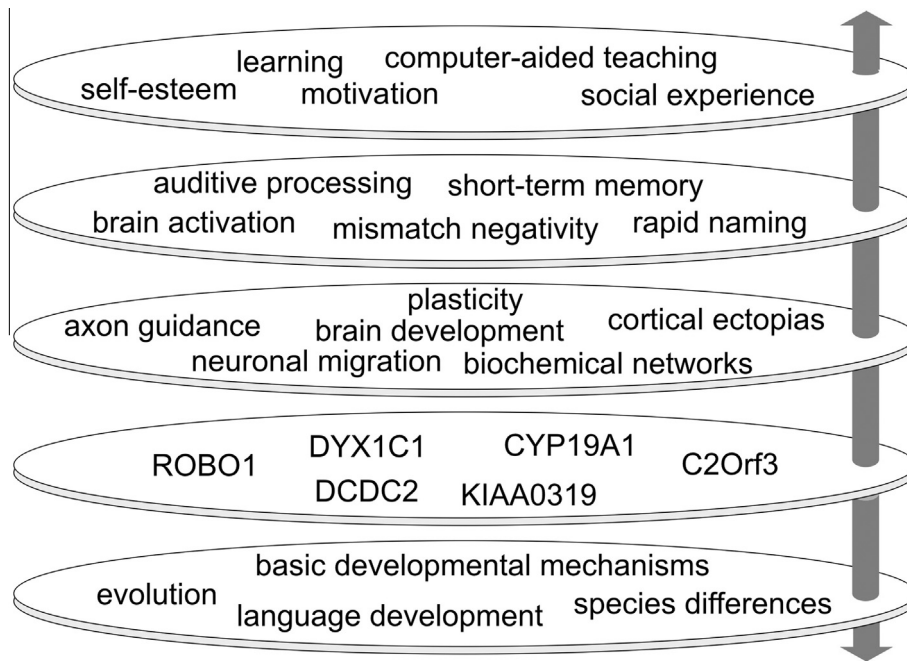


Fig. 2. DD is being studied and can be understood at multiple levels (from top to bottom), the social, neuropsychological, neurobiological, molecular genetic and evolutionary. Bridges are currently being built between neighboring levels. Our ability to connect through all the levels is still rudimentary.

with brain, neurological and renal anomalies, suggested a role in cilia-dependent interneuron migration [56]. Even arborization of dendrites by developing neocortical neurons was shown to depend on primary cilia [57].

These examples suggest that genes and proteins involved in cilia functions may have multiple, partly subtle effects in different organs, including in particular the brain. It is highly relevant to propose that DD may be considered a new type of ciliopathy, and that DD susceptibility genes, likely affecting neuronal migration and cortical morphogenesis may also have more dramatic pleiotropic effects in other ciliated organs, such as airways.

All these lines of research point toward DD as a disorder at the mild end of the spectrum of a number of pathways affecting developmental disturbances in neuronal positioning or connectivity. The severe end of the spectrum includes gross brain malformations that lead to deep mental retardation.

12. Conclusion

So far, attempts to genome-wide association studies have failed to implicate a single gene associated with DD. Nevertheless, in this complex disorder, early studies of rare individuals carrying chromosome abnormalities associated with DD as well as exceptional large families with monogenic inheritance patterns have suggested DD susceptibility loci and genes for functional studies. Even in the absence of strong association *p* values, the converging evidence from multiple lines of investigation support the conclusion that these genes and their implicated mechanisms are involved in regulating our ability to learn to read and write as well as causing DD when functionally compromised. Notably, however, the combined genetic effects of the genes identified so far fall short of explaining the strong genetic background in DD. Many more genes affecting the susceptibility for DD and development of our reading ability are likely to exist, and ongoing studies looking for more cases with extreme phenotypes may help in identifying new genes. They may reveal new mechanisms, or merge into the existing mechanisms that portray DD at the mild end of a spectrum of developmental

disturbances in neuronal positioning or connectivity. In any case, much further work will be needed to connect the multiple layers of understanding DD and ultimately the evolution of reading and writing skills (Fig. 2).

Acknowledgments

I wish to acknowledge the many contributions of my current and former laboratory members and collaborators. They are too many to be listed individually here. Our research to understand DD is supported by the Swedish Research Council, Swedish Brain Foundation (Hjärnfonden) and Sigrid Jusélius Foundation (Finland), and in the past by the Swedish Royal Bank Tercentennial Fund, Knut and Alice Wallenberg Foundation, and Academy of Finland.

References

- [1] H.C. Bastian, A Treatise on Aphasia and Other Speech Defects, H.K. Lewis, London, 1898.
- [2] M. Zahálková, V. Vrzał, E. Klobouková, Genetical investigations in dyslexia, *J. Med. Genet.* 9 (1972) 48–52.
- [3] B. Hallgren, Specific dyslexia (congenital word-blindness); a clinical and genetic study, *Acta Psychiatr. Neurol. Suppl.* 65 (1950) 1–287.
- [4] F.T. Cashell, Congenital dyslexia, *Proc. R. Soc. Med.* 62 (1969) 562–563.
- [5] R.K. Olson, Genes, environment, and dyslexia. The 2005 Norman Geschwind memorial lecture, *Ann. Dyslexia* 56 (2006) 205–238.
- [6] T. Fagerheim, P. Raeymaekers, F.E. Tønnessen, M. Pedersen, L. Tranebjærg, H.A. Lubs, A new gene (DYX3) for dyslexia is located on chromosome 2, *J. Med. Genet.* 36 (1999) 664–669.
- [7] J. Nopola-Hemmi, B. Myllyluoma, T. Haltia, M. Taipale, V. Ollikainen, T. Ahonen, A. Voutilainen, J. Kere, E. Widén, A dominant locus for developmental dyslexia in chromosome 3, *J. Med. Genet.* 38 (2001) 658–664.
- [8] T.K. Guttorm, P.H. Leppänen, A. Tolvanen, H. Lyytinen, Event-related potentials in newborns with and without familial risk for dyslexia: principal component analysis reveals differences between the groups, *J. Neural Transm.* 110 (2003) 1059–1074.
- [9] T.K. Guttorm, P.H. Leppänen, A.M. Poikkeus, K.M. Eklund, P. Lyytinen, H. Lyytinen, Brain event-related potentials (ERPs) measured at birth predict later language development in children with and without familial risk for dyslexia, *Cortex* 41 (2005) 291–303.
- [10] T. Klingberg, M. Hedehus, E. Temple, T. Salz, J.D. Gabrieli, M.E. Moseley, R.A. Poldrack, Microstructure of temporo-parietal white matter as a basis for reading ability: evidence from diffusion tensor magnetic resonance imaging, *Neuron* 25 (2000) 493–500.

- [11] E. Paulesu, J.F. Démonet, F. Fazio, E. McCrory, V. Chanoine, N. Brunswick, S.F. Cappa, G. Cossu, M. Habib, C.D. Frith, U. Frith, Dyslexia: cultural diversity and biological unity, *Science* 291 (2001) 2165–2167.
- [12] W.T. Siok, C.A. Perfetti, Z. Jin, L.H. Tan, Biological abnormality of impaired reading is constrained by culture, *Nature* 431 (2004) 71–76.
- [13] T. Scerri, G. Schulte-Körne, Genetics of developmental dyslexia, *Eur. Child Adolesc. Psychiatry* 19 (2010) 179–197.
- [14] J.D.E. Gabrieli, Dyslexia: a new synergy between education and cognitive neuroscience, *Science* 325 (2009) 280–283.
- [15] E.L. Grigorenko, Developmental dyslexia: an update on genes, brains, and environments, *J. Child Psychol. Psychiatr.* 42 (2001) 91–125.
- [16] J.F. Demonet, M.J. Taylor, Y. Chaix, Developmental dyslexia, *Lancet* 363 (2004) 1451–1460.
- [17] M. Taipale, N. Kaminen, J. Nopola-Hemmi, T. Haltia, B. Myllyluoma, H. Lyytinen, K. Müller, M. Kaaranen, P.J. Lindsberg, K. Hannula-Jouppi, J. Kere, A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 11553–11558.
- [18] K. Hannula-Jouppi, N. Kaminen-Ahola, M. Taipale, R. Eklund, J. Nopola-Hemmi, J.H. Kääriäinen, J. Kere, The axon guidance receptor gene *ROBO1* is a candidate gene for developmental dyslexia, *PLoS Genet.* 1 (2005) e50.
- [19] H. Meng, S.D. Smith, K. Hager, M. Held, J. Liu, R.K. Olson, B.F. Pennington, J.C. DeFries, J. Gelernter, T. O'Reilly-Pol, S. Somlo, P. Skudlarski, S.E. Shaywitz, B.A. Shaywitz, K. Marchione, Y. Wang, M. Paramasivam, J.J. LoTurco, G.P. Page, J.R. Gruen, *DCDC2* is associated with reading disability and modulates neuronal development in the brain, *Proc. Natl. Acad. Sci. U.S.A.* 102 (2005) 17053–17058.
- [20] J. Schumacher, H. Anthoni, F. Dahdouh, I.R. König, A.M. Hillmer, N. Kluck, M. Manthey, E. Plume, A. Warnke, H. Remschmidt, J. Hülsmann, S. Cichon, C.M. Lindgren, P. Propping, M. Zucchelli, A. Ziegler, M. Peyrard-Janvid, G. Schulte-Körne, M.M. Nöthen, J. Kere, Strong genetic evidence of *DCDC2* as a susceptibility gene for dyslexia, *Am. J. Hum. Genet.* 78 (2006) 52–62.
- [21] C. Francks, S. Paracchini, S.D. Smith, A.J. Richardson, T.S. Scerri, L.R. Cardon, A.J. Marlow, I.L. MacPhie, J. Walter, B.F. Pennington, S.E. Fisher, R.K. Olson, J.C. DeFries, J.F. Stein, A.P. Monaco, A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States, *Am. J. Hum. Genet.* 75 (2004) 1046–1058.
- [22] N. Cope, D. Harold, G. Hill, V. Moskvina, J. Stevenson, P. Holmans, M.J. Owen, M.C. O'Donovan, J. Williams, Strong evidence that *KIAA0319* on chromosome 6p is a susceptibility gene for developmental dyslexia, *Am. J. Hum. Genet.* 76 (2005) 581–591 (Erratum in: *Am. J. Hum. Genet.* 77 (2005) 898).
- [23] H. Anthoni, M. Zucchelli, H. Matsson, B. Müller-Myhsok, I. Fransson, J. Schumacher, S. Massinen, P. Onkamo, A. Warnke, H. Griesemann, P. Hoffmann, J. Nopola-Hemmi, H. Lyytinen, G. Schulte-Körne, J. Kere, M.M. Nöthen, M. Peyrard-Janvid, A locus on 2p12 containing the co-regulated *MRPL19* and *C2ORF3* genes is associated to dyslexia, *Hum. Mol. Genet.* 16 (2007) 667–677.
- [24] S.E. Fisher, C. Francks, A.J. Marlow, I.L. MacPhie, D.F. Newbury, L.R. Cardon, Y. Ishikawa-Brush, A.J. Richardson, J.B. Talcott, J. Gayán, R.K. Olson, B.F. Pennington, S.D. Smith, J.C. DeFries, J.F. Stein, A.P. Monaco, Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia, *Nat. Genet.* 30 (2002) 86–91.
- [25] T.S. Scerri, S. Paracchini, A. Morris, I.L. MacPhie, J. Talcott, J. Stein, S.D. Smith, B.F. Pennington, R.K. Olson, J.C. DeFries, A.P. Monaco, A.J. Richardson, Identification of candidate genes for dyslexia susceptibility on chromosome 18, *PLoS One* 5 (2010) e13712.
- [26] G. Poelmans, J.J. Engelen, J. Van Lent-Albrechts, H.J. Smeets, E. Schoenmakers, B. Franke, J.K. Buitelaar, M. Wuisman-Frerker, W. Erens, J. Steyaert, C. Schrander-Stumpel, Identification of novel dyslexia candidate genes through the analysis of a chromosomal deletion, *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 150B (2009) 140–147.
- [27] J. Nopola-Hemmi, M. Taipale, T. Haltia, A.E. Lehesjoki, A. Voutilainen, J. Kere, Two translocations of chromosome 15q associated with dyslexia, *J. Med. Genet.* 37 (2000) 771–775.
- [28] H. Anthoni, L.E. Sucheston, B.A. Lewis, I. Tapia-Páez, X. Fan, M. Zucchelli, M. Taipale, C.M. Stein, M.E. Hokkanen, E. Castrén, B.F. Pennington, S.D. Smith, R.K. Olson, J.B. Tomblin, G. Schulte-Körne, M. Nöthen, J. Schumacher, B. Müller-Myhsok, P. Hoffmann, J.W. Gilger, G.W. Hynd, J. Nopola-Hemmi, P.H. Leppänen, H. Lyytinen, J. Schoumans, M. Nordenskjöld, J. Spencer, D. Stanic, W.C. Boon, E. Simpson, S. Mäkelä, J.Å. Gustafsson, M. Peyrard-Janvid, S. Iyengar, J. Kere, The aromatase gene *CYP19A1*: several genetic and functional lines of evidence supporting a role in reading, speech and language, *Behav. Genet.* 42 (2012) 509–527.
- [29] K.E. Deffenbacher, J.B. Kenyon, D.M. Hoover, R.K. Olson, B.F. Pennington, J.C. DeFries, S.D. Smith, Refinement of the 6p21.3 quantitative trait locus influencing dyslexia: linkage and association analyses, *Hum. Genet.* 115 (2004) 128–138.
- [30] J. Nopola-Hemmi, B. Myllyluoma, A. Voutilainen, S. Leinonen, J. Kere, T. Ahonen, Familial dyslexia: neurocognitive and genetic correlation in a large Finnish family, *Dev. Med. Child Neurol.* 44 (2002) 580–586.
- [31] K.M. Stein, J.H. Schick, J. Gerry Taylor, L.D. Shriberg, C. Millard, A. Kundtz-Cluge, K. Russo, N. Minich, A. Hansen, L.A. Freebairn, R.C. Elston, B.A. Lewis, S.K. Iyengar, Pleiotropic effects of a chromosome 3 locus on speech-sound disorder and reading, *Am. J. Hum. Genet.* 74 (2004) 283–297.
- [32] K.L. Whitford, V. Marillat, E. Stein, C.S. Goodman, M. Tessier-Lavigne, A. Chédotal, A. Ghosh, Regulation of cortical dendrite development by Slit–Robo interactions, *Neuron* 33 (2002) 47–61.
- [33] W. Andrews, A. Liapi, C. Plachez, L. Camurri, J. Zhang, S. Mori, F. Murakami, J.G. Parnavelas, V. Sundaresan, L.J. Richards, *Robo1* regulates the development of major axon tracts and interneuron migration in the forebrain, *Development* 133 (2006) 2243–2252.
- [34] T.S. Scerri, F. Darki, D.F. Newbury, A.J. Whitehouse, M. Peyrard-Janvid, H. Matsson, Q.W. Ang, C.E. Pennell, S. Ring, J. Stein, A.P. Morris, A.P. Monaco, J. Kere, J.B. Talcott, T. Klingberg, S. Paracchini, The dyslexia candidate locus on 2p12 is associated with general cognitive ability and white matter structure, *PLoS One* 7 (2012) e50321.
- [35] Y. Wang, M. Paramasivam, A. Thomas, J. Bai, N. Kaminen-Ahola, et al., *DYX1C1* functions in neuronal migration in developing neocortex, *Neuroscience* 143 (2006) 515–522.
- [36] V.J. Peschansky, T.J. Burbridge, A.J. Volz, C. Fiondella, Z. Wissner-Gross, A.M. Galaburda, J.J. LoTurco, G.D. Rosen, The effect of variation in expression of the candidate dyslexia susceptibility gene homolog *Kiaa0319* on neuronal migration and dendritic morphology in the rat, *Cereb. Cortex* 20 (2010) 884–897.
- [37] A.M. Galaburda, G.F. Sherman, G.D. Rosen, F. Aboitiz, N. Geschwind, Developmental dyslexia: four consecutive patients with cortical anomalies, *Ann. Neurol.* 18 (1985) 222–233.
- [38] G.D. Rosen, J. Bai, Y. Wang, C.G. Fiondella, S.W. Threlkeld, J.J. LoTurco, A.M. Galaburda, Disruption of neuronal migration by RNAi of *Dyx1c1* results in neocortical and hippocampal malformations, *Cereb. Cortex* 17 (2007) 2562–2572.
- [39] S.W. Threlkeld, M.M. McClure, J. Bai, Y. Wang, J.J. LoTurco, G.D. Rosen, R.H. Fitch, Developmental disruptions and behavioral impairments in rats following in utero RNAi of *Dyx1c1*, *Brain Res. Bull.* 71 (2007) 508–514.
- [40] F. Darki, M. Peyrard-Janvid, H. Matsson, J. Kere, T. Klingberg, Three dyslexia susceptibility genes, *DYX1C1*, *DCDC2*, and *KIAA0319*, affect temporo-parietal white matter structure, *Biol. Psychiatry* 72 (2012) 671–676.
- [41] E. Paulesu, U. Frith, M. Snowling, A. Gallagher, J. Morton, R.S. Frackowiak, C.D. Frith, Is developmental dyslexia a disconnection syndrome? Evidence from PET scanning, *Brain* 119 (1996) 143–157.
- [42] S.A. Meda, J. Gelernter, J.R. Gruen, V.D. Calhoun, H. Meng, N.A. Cope, G.D. Pearlson, Polymorphism of *DCDC2* reveals differences in cortical morphology of healthy individuals – a preliminary voxel based morphometry study, *Brain Imaging Behav.* 2 (2008) 21–26.
- [43] T.C. Bates, P.A. Lind, M. Luciano, G.W. Montgomery, N.G. Martin, M.H. Wright, Dyslexia and *DYX1C1*: deficits in reading and spelling associated with a missense mutation, *Mol. Psychiatry* 15 (2010) 1190–1196.
- [44] S. Lamminenmäki, S. Massinen, J. Nopola-Hemmi, J. Kere, R. Hari, Human *ROBO1* regulates interaural interaction in auditory pathways, *J. Neurosci.* 32 (2012) 966–971.
- [45] I. Tapia-Páez, K. Tammimies, S. Massinen, A.L. Roy, J. Kere, The complex of *TFII-I*, *PARP1* and *SFPQ* proteins regulates *DYX1C1*, a candidate gene for dyslexia, *FASEB J.* 22 (2008) 3001–3009.
- [46] S. Massinen, K. Tammimies, I. Tapia-Páez, H. Matsson, M.E. Hokkanen, O. Söderberg, U. Landegren, E. Castrén, J.Å. Gustafsson, E. Treuter, J. Kere, Functional interaction of *DYX1C1* with estrogen receptors suggests involvement of hormonal pathways in dyslexia, *Hum. Mol. Genet.* 18 (2009) 2802–2812.
- [47] L. Wang, S. Andersson, M. Warner, J.Å. Gustafsson, Morphological abnormalities in the brains of estrogen receptor beta knockout mice, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 2792–2796.
- [48] L. Wang, S. Andersson, M. Warner, J.Å. Gustafsson, Estrogen receptor (ER)beta knockout mice reveal a role for ERbeta in migration of cortical neurons in the developing brain, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 703–708.
- [49] H.N. Fugger, T.C. Foster, J. Gustafsson, E.F. Rissman, Novel effects of estradiol and estrogen receptor alpha and beta on cognitive function, *Brain Res.* 883 (2000) 258–264.
- [50] F. Liu, M. Day, L.C. Muniz, D. Bitran, R. Arias, R. Revilla-Sanchez, S. Grauer, G. Zhang, C. Kelley, V. Pulito, A. Sung, R.F. Mervis, R. Navarra, W.D. Hirst, P.H. Reinhart, K.L. Marquis, S.J. Moss, M.N. Pangalos, N.J. Brandon, Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory, *Nat. Neurosci.* 11 (2008) 334–343.
- [51] S. Massinen, M.E. Hokkanen, H. Matsson, K. Tammimies, I. Tapia-Páez, V. Dahlström-Heuser, J. Kuja-Panula, J. Burghoorn, K.E. Jeppsson, P. Swoboda, M. Peyrard-Janvid, R. Toftgård, E. Castrén, J. Kere, Increased expression of the dyslexia candidate gene *DCDC2* affects length and signaling of primary cilia in neurons, *PLoS One* 6 (2011) e20580.
- [52] A.E. Ivliev, P.A. 't Hoen, W.M. van Roon-Mom, D.J. Peters, M.G. Sergeeva, Exploring the transcriptome of ciliated cells using in silico dissection of human tissues, *PLoS One* 7 (2012) e53618.
- [53] G. Chandrasekar, L. Vesterlund, K. Hultenby, I. Tapia-Páez, J. Kere, The zebrafish orthologue of the dyslexia candidate gene *DYX1C1* is essential for cilia growth and function, *PLoS One* 8 (2013) e63123.
- [54] A. Tarkar, N.T. Loges, C.E. Slagle, R. Francis, G.W. Dougherty, J.V. Tamayo, B. Shook, M. Cantino, D. Schwartz, C. Jahnke, H. Olbrich, C. Werner, J. Raidt, P. Pennekamp, M. Abouhamed, R. Hjej, G. Köhler, M. Gries, Y. Li, K. Lemke, N. Klena, X. Liu, G. Gabriel, K. Tobita, M. Jaspers, L.C. Morgan, A.J. Shapiro, S.J. Lettewboer, D.A. Mans, J.L. Carson, M.W. Leigh, W.E. Wolf, S. Chen, J.S. Lucas, A. Onoufriadi, V. Plagnol, M. Schmidts, K. Boldt, UK10K, R. Roepman, M.A. Zariwala, C.W. Lo, H.M. Mitchison, M.R. Knowles, R.D. Burdine, J.J. Loturco, H.

- Omran, DDX1C1 is required for axonemal dynein assembly and ciliary motility, *Nat. Genet.* 45 (2013) 995–1003.
- [55] M.A. Willaredt, K. Hasenpusch-Theil, H.A. Gardner, I. Kitanovic, V.C. Hirschfeld-Warneken, C.P. Gojak, K. Gorgas, C.L. Bradford, J. Spatz, S. Wölfl, T. Theil, K.L. Tucker, A crucial role for primary cilia in cortical morphogenesis, *J. Neurosci.* 28 (2008) 12887–12900.
- [56] H. Higginbotham, T.Y. Eom, L.E. Mariani, A. Bachleda, J. Hirt, V. Gukassyan, C.L. Cusack, C. Lai, T. Caspary, E.S. Anton, Arl13b in primary cilia regulates the migration and placement of interneurons in the developing cerebral cortex, *Dev. Cell* 23 (2012) 925–938.
- [57] S.M. Guadiana, S. Semple-Rowland, D. Daroszewski, I. Madorsky, J.J. Breunig, K. Mykytyn, M.R. Sarkisian, Arborization of dendrites by developing neocortical neurons is dependent on primary cilia and type 3 adenylyl cyclase, *J. Neurosci.* 33 (2013) 2626–2638.